encoding N-CAM-140 (Murray et al., 1986). The nature of the remaining extracellular coding region of the human muscle transmembrane protein isoform awaits further characterization of cDNA clones. The restriction maps of these further clones have some similarity with the 5' EcoRI fragment of λ1, but from restriction mapping there appears to be some sequence heterogeneity at the 5' ends of the cDNA clones.

To ascertain the mRNA transcript from which each cDNA clone was derived a Northern blot hybridization analysis was undertaken. Both the transmembrane and non-transmembrane probes hybridized to mRNA transcripts of 6.7, 5.2, 4.3 and 2.9 kb from a mixed human myoblast/myotube culture and of 7.2 and 6.7 kb from human embryonic brain tissue. Both probes failed to hybridize to any RNAs from human fibroblasts or lymphoblastoid cells. To relate cDNAs to the RNA transcripts of origin, specific subfragment probes were utilized. The transmembrane, 3' terminal fragment of clone A4.4 hybridized specifically to 6.7 and 6.7 and 7.2 kb transcripts from muscle and brain, respectively, whereas the 3' untranslated fragment of the full length clone hybridized to muscle transcripts of 5.2, 4.3 and 2.9 kb. Thus N-CAM transmembrane isoforms in muscle are encoded by a 6.7 kb transcript and by 6.7 and 7.2 kb transcripts in brain tissue. A probe derived from the MSD1 region only hybridized to non-transmembrane N-CAM isoforms from muscle cultures (5.2, 4.3 and 2.9 kb) and MSD1 was not expressed in any additional mouse brain or human brain transcript. The expression of MSD1 appears to be specific to muscle and its expression is restricted to myotubes, myofibres and denervated myofibres.

The isolation of cDNA clones encoding the full-length amino acid sequence of a human skeletal muscle non-transmembrane N-CAM isoform will now enable the development of expression models in vivo. Through study of these systems, the functional role of homophilic binding in myotube formation, innervation and reinnervation following neural injury may be clarified and in particular the role of the additional extracellular domains in muscle development may be defined.

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Molecular heterogeneity and differential expression of multiple protein kinase C subspecies in central nervous tissue

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Activation of protein kinase C (PKC) as a result of signal-induced diacylglycerol production, or directly, by exposure to synthetic permeable diacylglycerols or to tumour-promoting phorbol esters, has been implicated in the regulation of cell secretion, the modulation of membrane conductance, functional modification of receptors and other components of the signal transduction machinery, and the control of gene expression (reviewed by Nishizuka, 1984, 1986). PKC was purified to apparent homogeneity, and considered to be a single molecular entity until recent molecular cloning analysis predicted the enzyme to exist as a family of multiple subspecies, having closely related structures (Parker et al., 1986; Coussens et al., 1986; Ono et al., 1986a,b; Knopf et al., 1986; Makowske et al., 1986; Ohno et al., 1987; Housley et al., 1987). The relationship of the complementary DNA (cDNA) clones isolated in this laboratory to those of the
laboratories cited above has been discussed previously (Ono et al., 1987a; Kikkawa et al., 1987a). The complete primary structures of four subspecies, having α-, βI-, βII- and γ-cDNA sequences, have been elucidated by sequence analysis of the cDNAs obtained from a rat brain library (Kikkawa et al., 1987a, 1987b). In addition, chromatography on an hydroxyapatite column has permitted the resolution of purified samples of rat brain (Huang et al., 1986; Ono et al., 1987a; Kikkawa et al., 1987a) and rabbit brain PKC (Jaken & Kiley, 1987) into three separate protein kinase fractions. The correspondence of the rat brain enzymes isolated by this procedure to the PKC subspecies predicted on the basis of molecular cloning analysis was identified by transfecting the monkey kidney COS 7 cell line with plasmids containing the different cDNA sequences, followed by partial purification and resolution of the PKC subspecies (Kikkawa et al., 1987a). Type I PKC (the first peak of PKC activity eluting from the hydroxyapatite column, analogous to that of Huang et al., 1986) is encoded by γ-cDNA sequence, type II (second peak) is an unresolved mixture of two enzymes encoded by β- and βII-cDNA sequences, and type III (third peak) is encoded by α-cDNA sequence. Fig. 1 summarizes the common structural characteristics and features of these enzyme subspecies. The enzyme subspecies type I(γ), II(βI and βII) and III(α) are encoded by genes located on different chromosomes (Coussens et al., 1986), whereas type II(βII) and type II(βII) are derived from a single gene by alternative splicing of a common mRNA transcript (Ono et al., 1986b, 1987a). These subspecies have not yet been separated by conventional enzymological techniques, but have been studied by transfection of COS 7 cells with the respective cDNA-containing plasmids, and can be distinguished by polyclonal antisera raised against unique peptide sequences at the C-terminal end of the two subspecies (K. Ogita, unpublished work; Shearman et al., 1987; Ase et al., 1988). The two enzymes show similar sensitivities to Ca²⁺, diacylglycerol and phospholipid, and when purified from rat whole brain are present in the approximate ratio of 1:7.5. Each enzyme subspecies consists of four constant (C1-C4) and five variable (V1-V5) regions. Regions V1-V3 probably constitute the regulatory domain and C3-V5 the catalytic domain. The N-terminal end appears to be blocked. The C1 region contains a tandem repeat of six cysteine residues, which is analogous to the ‘zinc finger’ structural motif that is present in some DNA-binding proteins (Berg, 1986). Although all subspecies of PKC are dependent on calcium and phospholipid binding for their activity, no primary sequence within the regulatory domain offers itself as an obvious structure for this purpose. Knowledge of the secondary and tertiary structures of the enzyme molecules are thus needed to clarify this point.

The majority of PKC sequences reported in the literature can be categorized into the above group. Housey et al. (1987), however, have reported a clone, RP16, which appears to be distinct from those described above, and recent work in this laboratory has detected the existence of four new cDNA sequences which resemble, but are different from, those described above (Ono et al., 1987b, 1988). It is clear then, that the extent of diversity of the PKC enzyme family is not fully recognized at present.

A differential regional pattern of expression of PKC in brain tissue was documented in the early studies of Blumberg and colleagues on the autoradiographic distribution of [3H]phorbol 12,13-dibutyrate (PDBu) binding in calf (Nagle et al., 1981) and mouse brain (Nagle & Blumberg, 1983), and later by Snyder and colleagues in rat brain (Worley et al., 1986a,b; 1987). Immunocytochemical studies with polyclonal antisera raised against a purified, unresolved mixture of PKC subspecies, suggested that the enzyme was present in discrete cellular compartments (Biraud et al., 1985; Wood et al., 1986). After the discovery of multiple PKC subspecies by cDNA sequence analysis, hybridization studies in situ using probes complementary to the different cDNA sequences revealed that some of the PKC transcripts exhibited a differential regional and cellular distribution in brain tissue (Brandt et al., 1987).

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![Fig. 1](image-url)  
**Fig. 1. The common structural characteristics of four subspecies of protein kinase C isolated from rat brain tissue**

Analysis of clones obtained from a rat brain cDNA library predicted the complete primary structures of four enzyme subspecies. Chromatography of a purified enzyme preparation from rat whole brain tissue on an hydroxyapatite column allowed the resolution of three distinct enzyme fractions. Type II enzyme is a mixture of two enzymes which derive from alternative splicing of a common mRNA transcript. V, variable region; C, constant region; c, cysteine residue; M, metal ion; G, glycine residue; K, lysine residue; X, any amino acid.
We have investigated the distribution, in discrete areas of rat central nervous tissue, of the four PKC subspecies described above, by biochemical and immunological analysis of partially purified and resolved subspecies, and by immunohistochemical studies with subspecies-selective monoclonal antibodies and polyclonal antisera. Table 1 shows the relative activity of the enzyme subspecies isolated from the cytosolic (soluble) component of a number of tissue areas. A distinct regional expression of the enzyme subspecies is apparent. Type II(y) enzyme is most prevalent in the cerebellum, whereas it is detected only in low amounts in the spinal cord (cervical region). Type II enzyme represents the highest percentage activity in all regions tested, with the exception of the cerebellum. As mentioned above, this enzyme is a mixture of two subspecies, encoded by BL and BL-cDNA sequences, which can be distinguished by subspecies-specific polyclonal antisera (Shearman et al., 1987; Ase et al., 1988), and quantified by Western blotting analysis. In all regions tested, the type II(BL) subspecies was the major enzyme, with the type II(BL) subspecies being present at particularly low levels in the cerebral cortex and hippocampus. Type III enzyme showed the least regional variation.

Monoclonal antibodies were raised which were found to react selectively with the type I(y) enzyme, the type II BL and BL enzyme and one which reacts equally with all three enzyme fractions. Using a mixture of these antibodies, protein-kinase-C-like immunoreactive material was found to be discretely localized and primarily associated with neurons in a large number of areas of the rat central nervous system (Kitano et al., 1987; Saito et al., 1988). Using the monoclonal antibody selective for the type I(y) enzyme, and the polyclonal antisera against the type II BL enzyme, immunohistochemical analysis of the rat cerebellar cortex showed a clear cell-specific expression of these two enzymes (Fig. 2). Type I(y) enzyme is present in the Purkinje cell bodies, dendrites (which extend into the molecular layer) and axons (not clearly visible in the section shown in the Figure), whereas type II BL enzyme is present in the granular layer, where it is mainly associated with granule cell bodies. These unique staining features represent an example of differential enzyme expression which is also apparent in other regions of the brain, and also in non-neuronal cell populations such as lymphocytes (N. Berry, unpublished work). A similar such finding in the rat brain has also been reported by Mochly-Rosen et al. (1987), who have obtained monoclonal antibodies against a purified, unresolved mixture of protein kinase C, which specifically react with neuronal and/or astroglial cell populations in an area-dependent manner. These antibodies show some characteristics similar to the ones described in this paper, yet also exhibit distinct variations. Clearly, the use of a panel of antibodies directed against different epitopes will be necessary to obtain an overall, ‘processed’ picture of the distribution of the PKC subspecies.

This paper has briefly described the molecular heterogeneity and differential regional and cellular expression of PKC subspecies, that is apparent in central nervous tissue. When considered in conjunction with recent experimental findings which show that the enzyme subspecies isolated from brain tissue possess individual biochemical properties and a characteristic mode of activation (Huang et al., 1986; Jakon & Kiley, 1987; Sekiguchi et al., 1987; Sekiguchi et al., 1988), then a rational basis presents itself for reinterpreting, and attempting to explain the diverse physiological roles of PKC that have been reported in many different cell types. Clearly, it is essential to use the appropriate resolved enzyme subspecies in future functional studies.

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Table 1. Relative distribution of protein kinase C subspecies in the cytosolic fraction of different rat central nervous tissue areas

<table>
<thead>
<tr>
<th>Enzyme...</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Activity (% total cytosolic activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cDNA clone</td>
<td>γ</td>
<td>βI</td>
<td>βII</td>
<td>α</td>
</tr>
<tr>
<td>Whole brain</td>
<td>26.1</td>
<td>5.5</td>
<td>43.6</td>
<td>24.8</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>52.1</td>
<td>7.9</td>
<td>25.6</td>
<td>14.4</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>19.8</td>
<td>1.8</td>
<td>60.9</td>
<td>17.6</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>24.9</td>
<td>0.9</td>
<td>39.6</td>
<td>34.8</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>18.3</td>
<td>9.9</td>
<td>34.2</td>
<td>37.7</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>3.2</td>
<td>15.4</td>
<td>34.3</td>
<td>47.2</td>
</tr>
</tbody>
</table>

Fig. 2. Microphotographs of rat cerebellar cortex stained with antibodies against type I(y) and type II(BL) subspecies of protein kinase C.

Cerebellar sections were prepared as described by Saito et al. (1988), and stained by the peroxidase-antiperoxidase method. ML, molecular layer; PL, Purkinje cell layer; GL, granule cell layer.
Cloning of putative nicotinic acetylcholine receptor genes from the locust

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The nicotinic acetylcholine receptor (nACHR) in the vertebrate central nervous system (CNS) is encoded by a family of genes the sequences of which are homologous to those encoding the vertebrate muscle nACHR (Goldman et al., 1987). The CNS of insects also contains nACHRs, but in much greater concentrations than in the vertebrate CNS (Filbin et al., 1983). Thus, the insect is a very attractive system for the study of such neuronal receptors. This paper describes the isolation of several putative nACHR genes from the locust. The DNA of one of these has been partially sequenced and shown to contain an open reading frame that encodes part of a polypeptide that exhibits extensive amino-acid homology to known neuronal nACHR subunits.

Recently it has been reported that the nACHR in the CNS of insects is an oligomeric protein that is comprised of a single subunit type, M, 65,000 (Breer et al., 1984; Sattelle & Breer, 1985), which can be reconstituted in a planar lipid bilayer (Hanke & Breer, 1986) with recovery of cation-channel activity. In contrast, the evidence of MacAllan & Lunt (1986) suggests that the receptor from locust ganglia contains four different subunits and that the affinity ligand 4-[(N-maleimide)-benzyltrimethylammonium (MBTA) pre-dominantly labels a subunit of M, 49,000. These conflicting results give an indication of the difficulties involved in the purification and characterization of this receptor. To date, only one cDNA, namely that encoding a subunit of the Drosophila neuronal nACHR (as deduced by its amino-acid homology to vertebrate nACHR subunit sequences), has been cloned from insects (Hermans-Borgmeyer et al., 1986). However, the oligomeric structure of the receptor to which this subunit belongs, is not yet known. To circumvent the inconsistencies in the protein biochemical studies, we have been employing a gene cloning approach to investigate the structure of this receptor from the locust.

An amplified locust genomic library, constructed in λEM-1 (Karn et al., 1984), was screened using a 312-bp cDNA probe (λV16) which encodes part (from just within transmembrane helix M3) of a chicken brain nACHR subunit (Barnard et al., 1986). The screening was performed using hybridization conditions in which approximately 55% or greater homology at the DNA level should be detected. Out of 200,000 recombinants screened, one clone (gARL1) gave a positive signal. This clone also hybridized weakly with a 45-base 'best-guess' oligonucleotide probe (A. A. Hicks, M. G. Darlison & E. A. Barnard, unpublished work) which was synthesized to correspond to amino-acid residues 132–146 (Torpedo nACHR α-subunit numbering) of nACHR subunits. The DNA sequence encoding this region of nACHR subunits has been shown (Buonanno et al., 1986) to be highly conserved between species and between subunit types.

A fragment of the genomic clone that hybridized with both probes was isolated and subjected to DNA sequence analysis. The deduced amino-acid sequence of part of this...